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ORIGINAL ARTICLE

Is pseudarthrosis after spinal instrumentation caused by a chronic infection?

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Abstract

Hypothesis To assess whether a chronic bacterial infection is present in a subset of patients with pseudarthrosis after instrumented spinal fusion.

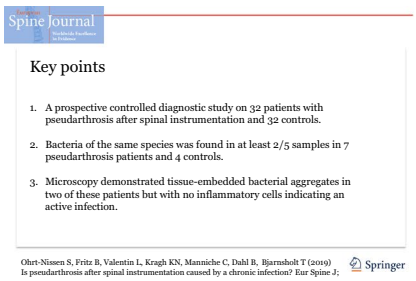
Methods This was a prospective diagnostic study including adult patients with previous instrumented spinal fusion. Patients underwent revision surgery for either pseudarthrosis or other causes (e.g. implant removal, curve progression or junctional kyphosis) (control group). Five separate biopsies were randomly collected, intraoperatively, from the pseudarthrosis site and cultivated under both aerobic (5 days) and anaerobic (14 days) conditions. If cultivation was positive in at least 2/5 tissue samples, the biopsy was sectioned and stained using peptide nucleic acid fluorescence in situ hybridization (PNA-FISH). Confocal laser scanning microscopy was used to examine the sections and visualize bacterial aggregates.

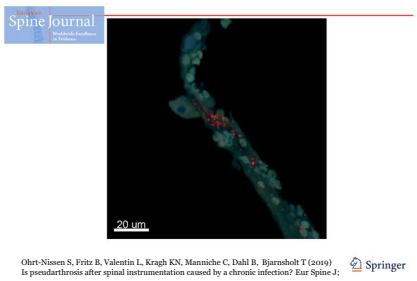
Results The study included 32 pseudarthrosis and 32 control patients. Cultivation yielded bacteria in at least 1/5 biopsies in 52% of patients with no difference between the groups ($p = 1.0$). Bacteria of the same species was found in at least 2/5 samples in seven pseudarthrosis patients and four controls ($p = 0.509$). *Propionibacterium acnes* was found in 8 of these 11 samples. Microscopy demonstrated tissue-embedded bacterial aggregates in two of these patients but with no inflammatory cells indicating an active infection. The presence of bacteria was not associated with the number of previous spinal procedures or the pre-revision fusion length ($p \geq 0.503$).

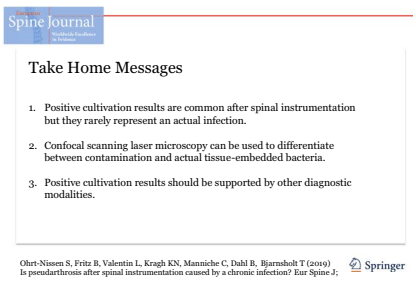
Conclusions Pseudarthrosis after instrumented spinal surgery was not significantly associated with the presence of bacteria at the pseudarthrosis site. Positive cultivation results are common after spinal instrumentation, but our results indicate that they rarely represent an organized infection.

Graphical abstract

These slides can be retrieved under Electronic Supplementary Material.







Keywords Pseudarthrosis · Non-union · Spinal instrumentation · Adult spinal deformity · Infection

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Extended author information available on the last page of the article

Introduction

Spinal instrumentation and fusion is performed on a variety of indications such as spinal trauma, degenerative conditions including deformity and paediatric spinal pathologies. The use of auto- and allografts is still a cornerstone in a successful spinal fusion and, despite significant development in spinal instrumentation and surgical techniques, pseudarthrosis remains a frequent problem. It is estimated that pseudarthrosis is the main indication in 25–40% of spinal revision surgeries [1, 2]. Pseudarthrosis refers to a failure of osseous union of the intended fusion area and results in persistent pathological segmental motion, which eventually leads to rod breakage and/or screw loosening. Patients typically present with persistent back pain and occasionally report a "snap" as the rod breaks. Standard radiographs are unreliable for detecting failure of osseous union, but rod breakage will appear on a standard X-ray of the spine. A CT scan can reliably verify the lack of fusion [3]. The aetiology behind the condition is poorly understood, and efforts to elucidate causative factors have been limited. Potential risk factors may be smoking, long-segment fusion, sagittal imbalance and pre-existing osteoarthritis [4].

The infection rate after spinal instrumentation is 2–3% [2, 5], and the clinical symptoms of chronic infection range from non-specific, monosymptomatic back pain to fistulation, fever and sepsis [6]. Subclinical infections with low-virulent bacteria such as *Propionibacterium acnes, Coagulase-negative staphylococci and Corynebacterium* spp. have recently gained attention due to their association with foreign device-related infections [7]. Dapunt et al. [8] found bacteria in the implant sonication fluid from 57% of patients with pseudarthrosis after surgically treated, long-bone fracture. However, the control group also yielded 40% cultivation-positive samples. The most frequently isolated bacteria were *P. acnes* and coagulase-negative *Staphylococcus* spp. *Propionibacterium acnes* is a slow-growing, aerotolerant, anaerobic gram-positive bacteria that has been linked to discitis, spondylodiscitis, osteomyelitis and paravertebral infection following surgical procedures [6, 9]. Diagnosing chronic infections of the spine with low-virulence bacteria is challenging and requires several peri-implant biopsies and prolonged cultivation. However, this approach also increases the risk of false-positive results, as a certain level of sample contamination is unavoidable. Physicians are often presented with inconclusive cultivation results, making it challenging to determine an appropriate treatment strategy.

While latent chronic infections have been linked to pseudarthrosis of long-bone fractures and other areas of bone surgery [8, 10], a microbiological causation for spinal pseudarthrosis has never previously been examined. As such, the purpose of the study was to assess whether a chronic bacterial infection is present in a subset of patients with pseudarthrosis after instrumented spinal fusion.

Methods

A prospective, single-centre, diagnostic trial was conducted between October 2015 through August 2017. All patients provided signed, informed consent prior to enrolment. Approval from the Ethical committee (H-6-2014-024) and the Danish Data Protection Agency (2007-41-1506) was obtained prior to the study.

Study population

We included a consecutive series of patients aged ≥ 18 years with previous spinal instrumentation undergoing revision surgery. All patients underwent revision using a posterior approach (PLF). Patients had no history of infection or wound-related issues (e.g. fistulation or late wound healing) nor any current clinical signs of infection. Based on the study by Chun et al. [11], patients were categorized as having pseudarthrosis when the following criteria were met: (1) a preoperative CT scan showing signs of fusion defect in the previously instrumented area and (2) intraoperative confirmation of pseudarthrosis with evident motion of the same segment with clear lack of fusion. Motion was tested by applying stress to the two vertebra adjacent to the pseudarthrosis by which clear motion between the two indicates lack of fusion. The control group consisted of patients undergoing spinal revision surgery for a variety of reasons including implant removal due to persistent pain, curve progression, junctional kyphosis or imbalance. Pseudarthrosis was ruled out intraoperatively. Exclusion criteria were more than 2 weeks of antibiotic treatment within 6 months prior to the current revision surgery, ongoing cancer or ongoing steroid-suppressive or other immunosuppressive treatment.

Sampling

All the sampling was performed in the sterile environment of the operating room. Five separate tissue biopsies were randomly collected, intraoperatively, from the site of the pseudarthrosis. The overlying scar tissue was discarded, and sampling was done at the centre of the pseudarthrosis site. In the control group, five biopsies were randomly taken from the soft tissue surrounding the instrumentation. All samples were placed in separate, sterile containers and sent for cultivation under aerobic (5 days) and anaerobic (14 days) conditions [12]. One additional biopsy was placed in a cryotube containing 10% formalin. In both groups, a 50-mm piece of

the rod was placed in a sterile container. The rod was treated with ultrasound sonication to loosen bacterial aggregates from the implant prior to cultivation of the sonication fluid. C-reactive protein and white blood cell counts were not routinely measured preoperatively as they have been shown to have little clinical value in low-virulence infections [13, 14].

Microscopy

If cultivation yielded bacteria of the same species in at least two samples, infection was considered probable and microscopic analysis using peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) was conducted. Using general histological preparation techniques, formalin-fixed tissue was embedded in paraffin, cut into 3–5-µm-thick sections and placed on adhesive glass slides. Sections were then deparaffinated and rehydrated using a xylene/ethanol series (2×xylene 5 min, 2×99% EtOH 3 min, 2×96% EtOH 3 min, 3×MilliQ Ultrapure H_2O 3 min). One drop of each *P. acnes*-specific and unibacterial PNA-FISH probe was added to the slide (Advandx, Boston, USA). A cover slip was placed on the slide and incubated at 55 °C for 90 min. The cover slip was removed, and the slide was then incubated for 30 min at 55 °C in a 1:60 dilution of 60x Advandx washing solution. After drying, sections were then counterstained using 3 µM 4′,6-diamidino-2-phenylindole (DAPI) for 25 min to visualize host cells [15]. Four sections were analysed per patient. The sections were visualized on a LSM 710 confocal laser scanning microscope (CLSM) (Zeiss, Jena, Germany) using a 63×1.4 (numerical aperture) oil immersion objective. Each section was analysed by scanning side to side over the entire piece of tissue. Bacterial aggregates were noted when the following criteria were met [16]:

(1) Bacteria organized in one or more aggregates, not as individual cells.
(2) Aggregates embedded at least 2 µm within the tissue.
(3) Inflammatory cells present in the tissue (either multilobed nuclei and/or increased concentration of normal nuclei in proximity to bacterial aggregates) indicating a host immune response.

For each patient, four separate tissue sections were analysed. All analyses were done by the second author who was blinded to the patient's diagnosis.

All patients were treated according to the same antibiotic protocol; after samples were obtained, 1.5 g of cefuroxime was administered i.v. Postoperative antibiotics were administered at the discretion of the surgeon. We recorded all patients receiving more than 3 days of postoperative antibiotic treatment. We recorded the 90-day infection-related readmission rate. We had access to all hospital medical charts in the capital region but were not necessarily advised if the patients were treated by their general practitioner.

Statistics

All statistical analyses were performed using R version 3.3.2 (R core team, 2014, Vienna). Data are reported as proportions (%) and mean with standard deviation (SD) or median with interquartile range (IQR). Data distribution was assessed by histograms and compared using Student's *t* test or Wilcoxon rank sum test depending on distribution. Categorical variables were compared using Pearson's Chi-squared test or Fisher's exact test.

Results

The study included 32 patients with pseudarthrosis and 32 control patients. Mean age was 62.9 (SD: 15.4), and 64% were female with no significant difference between the groups ($p \geq 0.297$) (Table 1). Two patients in each group were type II diabetics. The median number of spinal interventions prior to the studied procedure was 4 (IQR: 2–5) in the pseudarthrosis group and 2 (IQR: 1–6) in the control group. The length of fusion prior to the current revision was 13 [10–15] versus 5 [4–9] ($p < 0.001$). In both groups, the most frequent diagnosis at index procedure was adult degenerative deformity (Table 1).

Cultivation yielded bacteria in one or more samples in 16 pseudarthrosis patients and 17 controls ($p = 1.0$). Cultivation was monomicrobial in 29 cases (85%) and polymicrobial in five (15%).

Bacteria of the same species was found in at least two samples in seven pseudarthrosis patients and four controls ($p = 0.509$). *Propionibacterium acnes* was found in 8 of these 11 samples (Table 2). Microscopy demonstrated tissue-embedded bacterial aggregates in two patients (Figs. 1 and 2); however, no inflammatory cells could be visualized in proximity to the aggregates.

We found no association between the presence of bacteria in operative samples and the number of previous spinal procedures or the pre-revision fusion length ($p \geq 0.503$).

Three patients developed a postoperative infection within 90 days. Two had a superficial wound infection, and it was treated with oral antibiotics. One patient developed a fistula and was reoperated. None of these three patients originally had positive intraoperative cultures. One patient developed a fistula and was reoperated. Five patients had prolonged secretion from the surgical wound and received prophylactic antibiotics until wound closure (7–14 days). Two patients with *P. acnes* in 3/5 samples received 6 weeks of prophylactic antibiotics on the advice of our local department of microbiology. The patients had no clinical evidence of

Table 1 Demographic and clinical data

	Pseudarthrosis group	Control group	*p* value
Age at revision, median [IQR]	68.9 [53.2–71.0]	68.3 [50.6–73.2]	0.925
Female sex, no. (%)	23 (72)	18 (56)	0.297
No. of previous spinal procedures, median [IQR]	4 [2–5]	2 [1–6]	0.605
Pre-revision fusion length, median [IQR] no. vertebra	13 [10–15]	5 [4–9]	< 0.001
Principal diagnosis at index procedure, no. (%)			
Degenerative deformity	18 (56.3)	19 (59.4)	
Traumatic fracture	3 (9.4)	8 (25.0)	
Adult idiopathic scoliosis	7 (21.9)	3 (9.3)	
Congenital deformity	2 (6.3)	0 (0.0)	
Degenerative spondylosis	4 (12.5)	2 (6.3)	
Time since previous procedure			
Less than 12 months	3 (9.4)	5 (15.6)	
12–24 months	18 (56.2)	15 (46.9)	
More than 24 months	11 (34.4)	12 (37.5)	0.665

Table 2 Microorganisms cultured in cases and controls from both aerobic cultivation and anaerobic cultivation

	Pseudarthrosis group	Control group
Organism cultured in one sample or more, $n = 34$		
P. acnes	9	9
Coagulase-negative Staph spp.	5	4
Micrococci spp.	1	1
Gram-negative rods	0	1
P. avidum	1	0
Non-haemolytic streptococci	0	1
Arthrobacter spp.	0	1
Organism cultured in at least two samples from the same patient, $n = 11$		
P. acnes	5	3
Staph spp.	2	0
Micrococci spp.	0	1

infection, but due to the risk of biofilm formation on the inserted implant this approach was considered justifiable.

Discussion

Revision surgery after spinal instrumentation remains a significant challenge. Normal screening of blood samples and clinical evaluations are used to rule out infection but is not reliable for chronic biofilm-related infections. Biofilms often form around retained implants, and the hypothesis of the present study was that a subclinical biofilm infection was present on the spinal implants, which would ultimately disrupt bone formation leading to failure of a solid fusion and clinical pseudarthrosis. We did not find any evidence to support this hypothesis. In this prospective controlled study, we found a large proportion of cultivation-positive cases but with no difference between pseudarthrosis patients and controls. *Propionibacterium acnes* was the most frequently isolated organism. Leitner et al. [17] presented cultivation results from 110 cases of spinal metal explanation and identified bacteria in 29% of cases, most frequently *Staphylococcus and Propionii* spp. The authors found that patients with screw loosening had significantly higher rates of positive cultivation. Conversely, Shifflett et al. [18] obtained operative cultures in 112 cases of spinal revision. In an uncontrolled, retrospective design, the authors found positive cultures in 40% of cases and found that patients with pseudarthrosis had higher rates of positive cultivation. Callanan et al. [19] presented an uncontrolled case series on 43 patients with no signs of infection undergoing revision after previous instrumentation. They cultivated bacteria in 37% of cases with *P. acnes* being the most prevalent bacteria. Gelalis et al. [20] found a contamination rate of 20% of patients after randomly cultivating wound swaps. *Propionibacterium acnes* is a commensal organism of low virulence

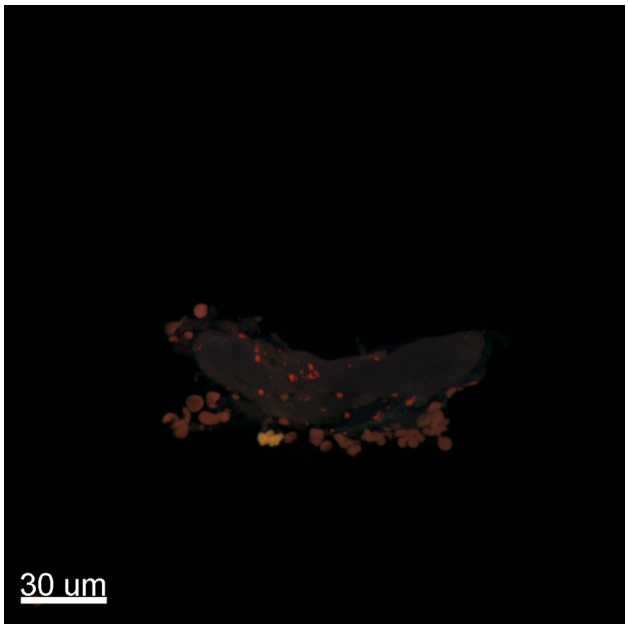

Fig. 1 Fluorescence in situ hybridization of tissue sample from a pseudarthrosis patient. Bacterial aggregates are seen embedded in the tissue. No inflammatory cells are seen

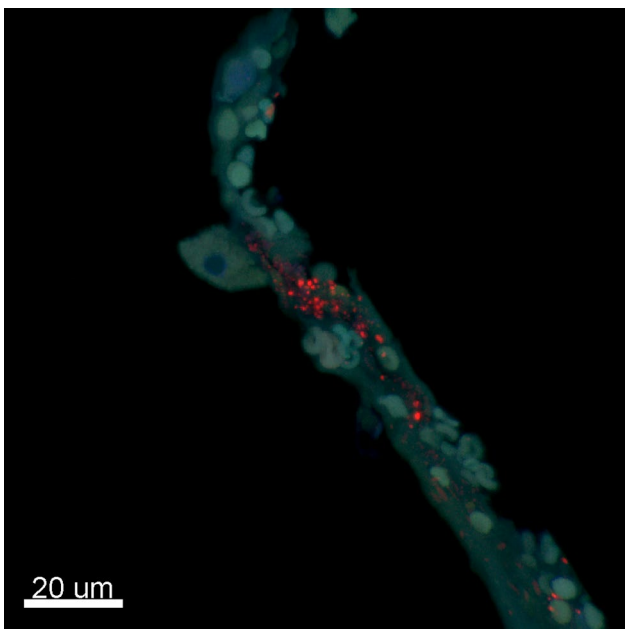

Fig. 2 Fluorescence in situ hybridization of soft tissue sample from a control patient undergoing implant removal after a thoracolumbar fracture. Bacterial aggregates are seen embedded in the tissue. No inflammatory cells are seen

that populates the dermal sebaceous glands, and positive cultures are difficult to interpret in patients with no obvious signs of infection [12, 21]. In the current study, cases with two or more positive cultures underwent further examination with PNA-FISH staining and CLSM on multiple tissue sections. Signs of bacterial aggregates were seen in two patients fulfilling the criteria of organized bacteria embedded in the tissue (Figs. 1 and 2). However, inflammatory cells could not be visualized in proximity to the aggregates. PNA-FISH analysis using CLSM has a high sensitivity and specificity for bacterial biofilms [22, 23], but is not a high throughput method as only a 5-µm-thick tissue section is examined. A previous study found that PNA-FISH analysis does not correspond well to biomolecular findings [16]. Biofilms spread heterogeneously throughout the tissue and may not appear in specific tissue sections. Furthermore, cultivation and biomolecular analysis (e.g. PCR) cannot discriminate between contamination and actual infection. We attempted to increase the sensitivity of our analysis by examining several tissue sections as well as sonicating explanted metal rods and culturing the fluid. Biofilms are heterogeneously distributed within tissues; thus, it is of great importance to sample multiple sites. It is not known exactly where bacteria are located during these infections, but sonication of explanted devices and homogenization of tissue may increase sensitivity [24–26].

Our study demonstrated a very high number of cultivation-positive samples, but in many cases only in one out of five samples. We found no evidence of tissue infiltration of bacteria or inflammatory cells. We hypothesize that sample contamination (in the operation room or in the subsequent handling) is a key factor to consider. Furthermore, while the implant may show signs of colonization this does not necessarily translate to a clinically relevant tissue infection. We do not consider a single positive cultivation without clinical or histological signs of infection to merit treatment in these patients. The recently published OVIVA trial [27] defined plausible tissue infection as two or more phenotypically indistinguishable bacteria from the same tissue. We consider this to be a reasonable approach, and using this definition the rate of "positive" cultivations in our study was considerably lower. Our study was not designed to identify patients who would benefit from routine sampling during revision surgery and whether positive samples require subsequent treatment. We could not identify a clinical consequence of the microbiological results. Therefore, we do not recommend routine sampling during surgical treatment of these patients until a subgroup of patients have been more appropriately identified. Unwarranted sampling will inevitably lead to false-positives and potentially unnecessary therapeutic implications. Two patients with positive cultivations received prolonged antibiotics to avoid postoperative biofilm formation on the advice of the department of microbiology. This treatment was not protocolized and was done on a case-by-case basis.

Previous studies have presented low-grade evidence suggesting a link between implant-related complications and a low-grade infection. These findings were

clinically interesting as they may imply the need for modified approaches of revision surgery, potentially involving two-stage surgery, intraoperative microscopy or postoperative antibiotic regimes. The current study found that cultivation-positive cases were common, but no specific association with pseudarthrosis could be found. We hypothesize that the insertion of spinal implants may involve colonization of low-virulence bacteria [28], but that the clinical implication remains to be elucidated. Future studies should incorporate additional diagnostic approaches other than cultivation to verify whether an actual infection is present.

Conclusions

Pseudarthrosis after instrumented spinal surgery was not significantly associated with the presence of bacteria at the pseudarthrosis site. Positive cultivation results are common after spinal instrumentation, but our results indicate that they rarely represent an organized infection. Positive cultivation results in these patients should be supported by other diagnostic modalities in the absence of clinical signs of infection.

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Compliance with ethical standards

Conflict of interest Benny Dahl received consultant fees outside the submitted work from K2 M.

Ethics approval Approval from the Ethical committee (H-6-2014-024) and the Danish Data Protection Agency (2007-41-1506) was obtained prior to the study.

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